Effect of Long Heat-Denaturation of Collagen on Its Electrophoretic Pattern

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For the denaturation of soluble collagen, Piez *et al.*[1] suggested heating at pH 4.8 for 15 min at +40°C. The detailed investigation by Engel [2] showed that the denaturation proceeds in two stages: the helical structures are lost very rapidly but the separation of the chains takes more time, and he suggested heating for 90 min for complete denaturation. In a later paper Engel and Beier [3] demonstrated that the α- and β-components are not stable, but already after 9 h at +40°C in pH 3.7 citrate buffer 10–20 % of the β-component was lost from the sedimentation pattern, and also degradation products of small molecular weight were obtained from both α- and β-fractions. These findings prompted us to check the electrophoretic pattern of heat-denatured collagen after various intervals.

The collagen preparation was obtained from rat tail tendon fibres by extraction for 24 h in the cold with 20 volumes of 3 % (v/v) acetic acid. (The material had been first extracted exhaustively with 0.4 M NaCl, buffered to pH 7.3 with 1/15 M phosphate, and the soluble fraction discarded.) The collagen was precipitated from the acetic acid extract by dialysis against pH 7.3, M/15 phosphate buffer. The precipitate was lyophilized, dissolved in 3 % acetic acid and dialyzed against the pH 4.5 acetate buffer, ionic strength μ = 0.022, which was also used in the preparation of the gel for the electrophoretic run.[4] For the prevention of microbial growth, the buffer was saturated with octanol. No turbidities were observed. After a preliminary denaturation at +40°C for 15 min, the sample was divided in separate lots, which were kept at +4°C, until they were immersed in a +40°C water bath at appropriate intervals to get the indicated heating times at the beginning of the simultaneous electrophoretic runs on the same gel sheet (Fig. 1, except the run marked 5 d–B.).

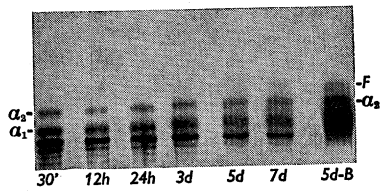

Fig. 1. Effect of long heat-denaturation of soluble collagen on the starch gel electrophoretic pattern. The duration of the denaturation at +40°C is indicated. The sample size was 400 μg, but in some runs larger samples were applied to accentuate the fast-moving fraction F (the pattern to the extreme right).

The pattern does not change essentially in a week, but three observations were made: (1) The slow-migrating fractions were gradually lost during the heating and they therefore represent larger complexes, which are broken down. (2) The α_1-fraction becomes rather broad. The separation to subcomponents is not clear, but on the basis of unpublished work by V. Näntö from our laboratory we believe that this fraction is not homogeneous. (3) After about 3 days there appears a new distinct band designated F, which migrates faster than the α_2-component. In Fig. 1 the

run 5d—B is included to demonstrate this fraction more clearly. Also in the preparative modification of the starch gel electrophoresis (unpublished work) there appears a fast-moving fraction of gelatin. The migration rate of the new band corresponds to that of the commercial gelatin obtained from limed precursors.[4] The possibility of an artefact from microbial growth does not seem likely to us, mainly because of the regular appearance and distinct form of the band, which has been observed also in the fractionation of other samples of denatured collagen.

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